Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol., No.6, 2015
www.iiste.org

Changes in Aortic Vasa Vasorum Associated with Rabbits Hyperimmunization with Pseudomonas Aeruginosa

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Abstract
Aortic adventitial vasa vasorum (vv) is an essential network of microvesseles that participates in providing nutrient , oxygen , besides being a source of stem cell for neovascularization (vv) is involved in inflammatory response in atherosclerosis . Male rabbits were immunized with heat killed , Whole bacteria, rested for two weeks and aortic base region were processed for histological examination . In addition the sex-steriods hormonal levle were estimated by ELISA . The results demonstrated that immunized rabbit showed prominent thickened tunica media with signs of smooth muscle cell proliferation . The most interesting findings included increased angiogenesis . Rabbits showing these changes demonstrated increased testosterone 5.77± 3.78 in test versus 1.25 ± 0.87 in control (P=0.089) . progesterone and estradiol didn't show any changes in test animals . These results implicate that contineous exposure to bacterial constituents could induce atherosclerotic lesion in aorta vasa vasorum.
Keywords: Vasa Vasorum (VV) , Immunization, Pseudomonas aeruginosa

Introduction
Adventitial vasa vasorum (vv) is a network of small blood vessels that provide large blood vessels, including aorta, nutrients and oxygen and also serve to remove wastes. The adventitial cells perform diverse functions and include fibroblast, dendritic cell, macrophage, progenator cells, endothelial cells of vasa vasorum, pericytes as well as other cells. The vasa vasorum is intimately involved in processes like vascular inflammation and vessel wall remodeling (Stenmark *et al* ., 2013). during inflammatory response within adventitia induced by vessel injury atheromatic microvessels vasa vasorum are increased (Kahlon *et al* ., 1992; Moulton, 2001) and this angiogenesis is involved in atherogenesis (Hu.Y&Q Xu, 2011). Adventitial inflammation occurres in adventitial vessels including vasa vasorum and this vessel could be a source of a panel of cytokines including TNF alpha , TGF Beta , G-CSF , GM-CSF, monocyte chemoattractant protein-1 (MCP-1) and others (Scotland *et al*., 2000). Lipopolysaccharide (LPS) of gram negative bacteria stimulates vascular smooth muscle cells (SMC)through TLR_4 pathway (Jiang *et al*., 2014). In this communication we wanted to see if chronic exposure represented by hypersimmunization of rabbits with a gram negative *Pseudomonas aeruginosa* could have an effect on the vital adventitial aortic vasa vasorum.

Material and Methods:
Animals
Domestic outbreed rabbits of both sexes were used, The age of animals range feom 4-6 months. They were housed in parirs and fed ad libidum with chow meal. They were ethically treated according to the established guidline in our department.
Hyperimmunization
Rabbits were hyperimmunizied, using a boiled, three times washed cell suspension of *Pseudomonas aeruogionsa* originaly isolated from stool. The procedure of (Duncan *et al*., 1976) was used with some modification including above neck subcutaneous route immunization. The animals were rested for 2 weeks and sacrified while they were under ketamine and xylocan ansthesia. Oarta were obtained, fixed with 10% formladhyde, processed, embeded in paraffin sections and stained with hematoxyline and eosine and examined for histological changes That occur in tunica adventitia, insisting on the change in vasa vasorum.
Hormonal levels
Steriod sex hormones including progestrone (p), Estradiol (E) and Testestrone (T) were estimated using commercial ELISA Kits , according to the procedure of the manufactuer.
Statistical analysis
Means ± standared deviation of different treatments values were obtained. Test versus control statistical differences were evaluated using t-test and Epidemiological statistics program . Data regarded significant at $P \leq 0.05$.

Results
All male rabbits hyperimmunized with *Pseudomonas aeruginosa* showed signs of atherosclerotic changes compared to non-immunized animals (Table 1). The prominent changes included thickened tunica media, media

smooth muscle cells(SMCs) proliferation in vasa vasorum reminescent of atherosclerosis (Figure1). The most interesting finding was increased vasa vasorum angiogenesis also (Figure 2).

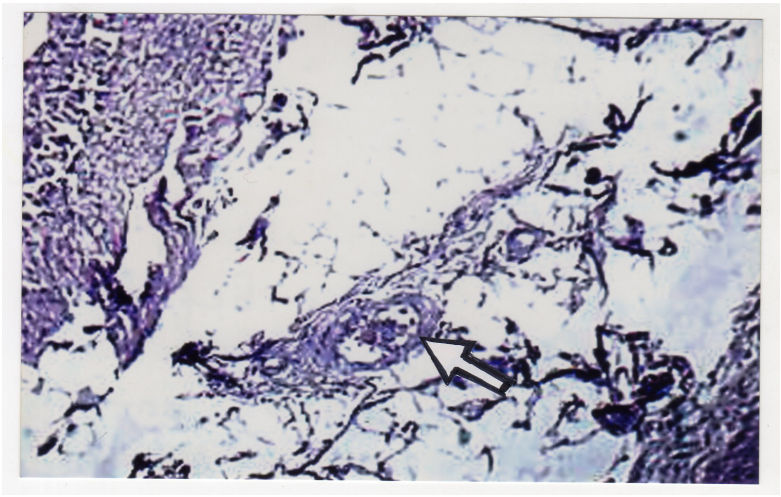
Table (1) . Effect of *Pseudomonas aeruginosa* hyperimmunization on aortic vasa vasorum angiogenesis in male rabbits and controls.

Angiogenesis		
Treatment	Positive / total	%
Test	5/5	100
Control	1/3	33

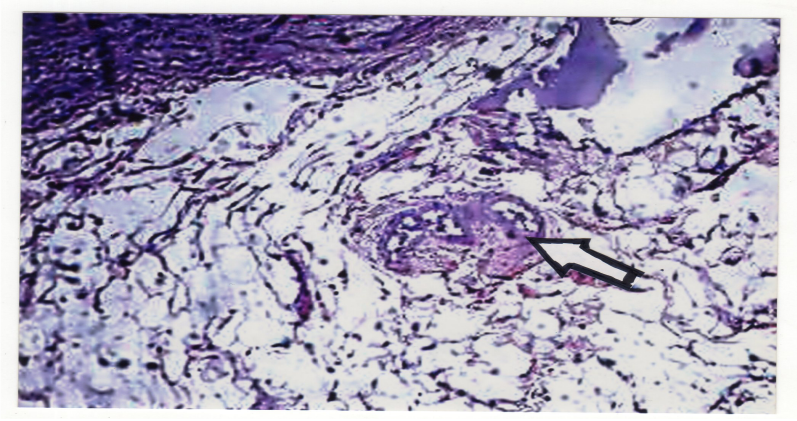
Table (2). Sex-hormones levels in male rabbits hyreimmunized with *Pseudomonas aeruginosa* and controls.

Hormone Leveles ng/ml (mean+ SD)			
Treatment	Progestrone	Estradiol	Testestrone
Test n=4	2.2±0.37*	21.05±9.5*	5.17±3.78**
Control n=4	2.13±0.31	18.2±4.08	1.25±0.86

*Not significant at $p \leq 0.05$ (T-test)
**P=0.089.



(a)



(b)

Figure 1: Section of rabbit Oarta .
(a) showing vasa vasorum (vv) of normal rabbits .
(b) (b) showing (vv) of test rabbits with atherosclerotic changes .Arrow point out to sclerotic changes . Original magnification.400x

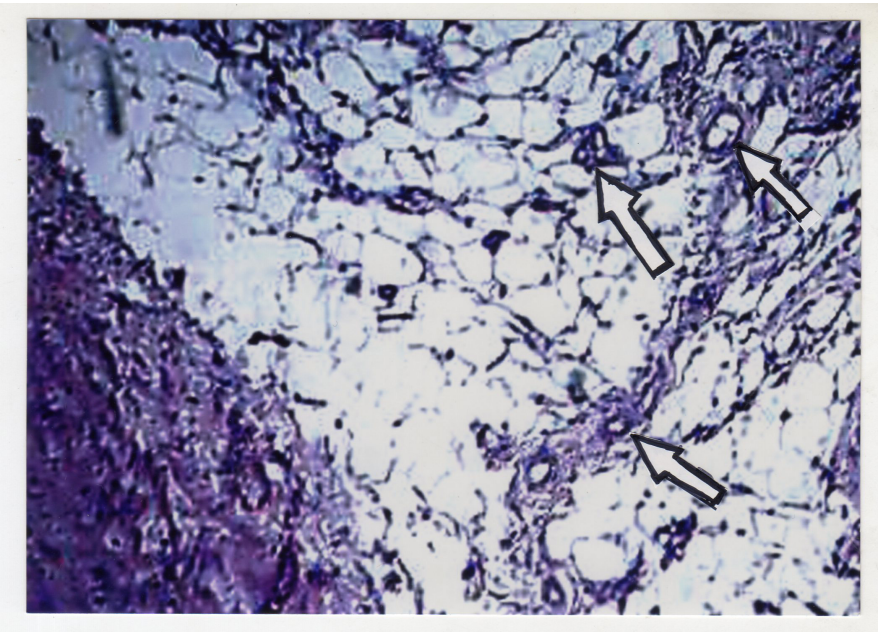


Figure 2: Section of rabbit Oarta , showing neumerous vasa vasorum (angiogenic change) of test rabbit. Original magnification 400x.

Discussion

The histslogical changes in tunica adventitia vasa vasorum that included thickened media layer seen in this report are features of atherosclerosis as seen in human (Ogeng'o *et al*., 2014). In addition the increasd vasa vasorum angiogensis in the adventitia promotes the growth of atherosclerotic plaques (Kawabe&N.Hasebe, 2014). It was seen that vasa vasorum in the adventitial layer has very important role in vessel inflammation (Maiellaro&W.R.Taylor,2007; Eriksson, 2011).

The role of chronic exposure to gram regative bacterial constituents including lipopolysaccharide emerged here as increased vasa vasorum angiogenesis might be mediated by the inflammatory response stimulated by LPS. ln this regard Kandasamy et al (Lecce *et al*., 2014) reported that LPS induced micorvessels inflammation through nuclear factor kappa B activation in lung microvessels. Supporting this notion, LPS was shown to induce vascular smooth muscle cells proliferation through TLR4 (Jiang *et al*., 2014).

The peculiar changes of increased (vv) angiogenesis require additional investigation. ln rabbits showing histopatholoigcal changes, there were a parallel hormonal changs seen in Testestrone level (Table 2).

The mean ± standared deviation of test animals was 5.17±3.78 versus 1.25± 0.86 in control group P=0.089 which might implicate testestrone mediated pathway in these effects.

On the other hand however, progestrone and estradiol didn't demonstrate any changes in test animals compared to control.

The significance of increased testestrone accompany hyper- immunization with *Pseudomonas aeruginosa* and increased angiogenesis are unclear at the present time. Nevertheless, emerging evidence indicates that androgen regulates angiogenesis(Lecce *et al*., 2014). It was also shown that testestrone promotes angiogenesis by enhancing expression of cytokines HIf-la, SDF-la and VEGF. This pro-angiogensis effect is mediated by CD34+ stem cell mobilization(Chen *et al*., 2012) .

In connection with this, adventitial multipotent pericyte is a structural entity of vasa vasorum thus implicate this microvessele as a reservoir for vascular stem cell and angiogenesis (Kawabe&N.Hasebe, 2014) .

The findings reported in this communication highlight a link between chronic exposure to a gram negative bacteria and atherosclerosis . ln depth investigation will shed light on the impact of this process in atherosclerosis.

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